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# Effects of Toxic Substances on Female Reproduction

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**Successful reproduction requires a complex series of interdependent physiological, cellular and molecular events. In the female many of these interdependent events are vulnerable to interruption by xenobiotic compounds. The physiological steps in the female reproductive cycle are reviewed. Selected xenobiotics which interrupt this cycle are presented and their mechanisms and site of adverse effects are discussed. Finally, a more detailed discussion of chemically induced ovarian failure in the human and an experimental animal model system is presented.**

## Introduction

Successful reproduction in the female requires the stepwise completion of a complex series of interdependent events (Fig. 1). Existing evidence suggests that this reproductive cycle is vulnerable at many points to the adverse influence of zenobiotic substances, including drugs and environmental pollutants (*1-6*). Two additional events not addressed in Figure 1 which must be considered in a discussion of female reproductive toxicology are the age of female reproductive senescence and the control of female fertility.

As the length of time between sexual maturation and reproductive senescence directly influences the potential reproductive capacity of a female, xenobiotics which alter the age of sexual maturation or reproductive senescence may have a profound impact on human reproduction and well-being. At the present time several drugs, biotics and xenobiotics have been identified which will produce premature senescence of the female reproductive system (*3-5, 7-9*). Prepubertal or prenatal exposure to these compounds may also prevent the onset of puberty as a direct result of their toxicity (*10-14*).

The expression of fertility in human populations has considerably greater significance than just successful functioning of the reproductive system. The offspring, products of this system, represent an economic and temporal responsibility for the parents. Because of this, considerable effort has been invested in the development of safe and reliable methods of contraception. Another area of concern in female reproductive toxicity is therefore the identification and characterization of xenobiotics which impair the mechanism of action of contraceptives. This is especially crucial in situations where women are exposed to complex mixtures containing

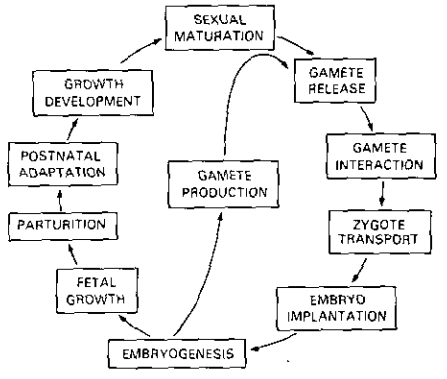


FIGURE 1. Reproductive cycle in the female. This figure represents a summary of the physiological events required for successful reproduction. Reprinted from Mattison (*4*) with permission.

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compounds which decrease the effectiveness of their chosen contraceptive, and others which act as teratogens.

Because reproductive toxicology encompasses reproductive biology and toxicology it is necessary to consider both the mechanism and site of action of a reproductive toxin. This dual approach is required because of: strain and species differences in reproductive biology; strain and species differences in toxification, detoxification, and repair of cellular damage; strain and species differences in sensitive windows for reproductive toxicity; and strain and species differences in reproductive end points monitored to assess toxicity. To approach this perspective we will briefly consider the events necessary for successful female reproduction and identify several xenobiotics which interrupt reproduction at specific sites. We will then present a systematic approach to organize and understand the diverse mechanisms of these reproductive toxins. Finally, we will consider in some detail the mechanism of action of xenobiotics which produce premature ovarian failure.

## Female Reproduction

The reproductive events outlined on Figure 1 can be arbitrarily divided in many ways, one clinically and experimentally useful division is shown on Table 1. This framework will allow an overview of female reproduction and introduction of selected reproductive toxins.

**Table 1. Female reproduction.**

| |
|---|
| Prefertilization |
| Reproductive system development |
| Sexual maturation |
| Ovulation-meiosis-corpus luteum |
| Hormonal effects |
| Ovum transport |
| Sperm transport |
| Fertilization to implantation |
| Sperm-egg recognition |
| Sperm penetration |
| Sperm nuclear decondensation |
| Pronuclear interaction |
| Conceptus transport |
| Conceptus-endometrium interaction |
| Placentation to parturition |
| Endometrial development |
| Cytotropoblast-syncytiotrophoblast |
| Placental growth-maturation |
| Fetal organogenesis |
| Fetal growth-maturation |
| Maternal metabolic alteration |
| Fetal-placental-maternal interaction |
| Parturition |
| Fetal-maternal interaction |
| Uterine electrical and muscular activity |
| Cervical relaxation |
| Birth canal |
| Postnatal |
| Placental delivery |
| Uterine involution |
| Fetal adaptation |
| Lactation |
| Maternal metabolic adaptation |
| Reproductive senescence |
| Hypothalamic-pituitary failure |
| Ovarian failure |
| Uterine failure |

### Prefertilization

The reproductive processes grouped under prefertilization are quite broad and include development of the reproductive system, sexual maturation, gamete release and transport, as well as hormonal effects on reproductive organs. Xenobiotics which alter development of the reproductive organs may have a profound effect on subsequent reproductive function. Several xenobiotic compounds have been identified which alter development of the reproductive system including; diethylstilbesterol, certain polycyclic aromatic hydrocarbons, purine analogs (azathioprine and 6-mercaptopurine) and galactose.

Diethylstilbesterol (DES) is interesting because it embodies a mechanistic dilemma and illustrates the often cryptic nature of reproductive toxins. DES was introduced into obstetrical practice in the early 1940s as a therapeutic modality for pregnancy wastage. The rationale and effectiveness have been previously reviewed (4). It was subsequently discovered that this treatment produced vaginal adenocarcinoma in less than 1/1000 women exposed *in utero* during the first trimester.

Additional and continuing studies have attempted to characterize the effects of DES on the structure and dynamic functioning of the female reproductive system. These studies suggest that prenatal DES treatment alters both the development and function of the female reproductive system (4). In spite of the captive nature of the exposed population and the seemingly well-defined exposure, considerable disagreement exists regarding the actual reproductive toxicity of prenatal DES exposure. This disagreement appears to result in part from the broad therapeutic protocols used in prescribing the drug. Patients and controls with well-documented treatment during the first trimester, such as the Chicago Lying-In double-blind study, may be the best group to explore the toxicity of DES.

Galactose and the purine analogs, azathioprine and 6-mercaptopurine, also alter reproductive system development in a very specific way. All three

compounds produce ovarian toxicity by blocking some facet of oogenesis (*14-16*).

Sexual maturation can be altered by several xenobiotics. Compounds which block oogenesis or destroy oocytes before puberty leaving the ovary devoid of follicles will clearly block pubertal progression. Estrogen agonists in sufficiently high doses will produce a precocious pseudopuberty; this may also occur with certain halogenated polycyclic hydrocarbons (*17-21*) and phytoestrogens (*22*).

Ovulation, resumption of meiosis, and corpus luteum function as well as secondary hormonal effects during the menstrual cycle depend on dynamic hormonal interactions along with hypothalamic-pituitary-ovarian-uterine axis (*23*). Xenobiotics which interrupt these dynamic interactions include pesticides, barbiturates, polycyclic aromatic hydrocarbons, halogenated aromatic hydrocarbons, and steroid hormone agonists and antagonists (*3,24*). Although compounds which interrupt these dynamic endocrine feedback processes are generally thought to act only during the time of exposure, there is suggestive evidence that prenatal exposure to certain pesticides may alter subsequent functioning of the hypothalamus and pituitary (*3,24*).

The effects of xenobiotics on ovum and sperm transport are poorly understood. Further research is needed to define the physiological and cellular mechanisms controlling these processes. At the present time the major factors associated with disordered sperm, ovum and conceptus transport appear to be sequelae of uterine and tubal infections and endometriosis.

## Fertilization to Implantation

The complex endocrine, cellular and molecular events involved in mammalian gamete recognition, sperm penetration, nuclear decondensation and pronuclear interaction are presently under investigation. The effects of xenobiotics on these processes remain poorly characterized.

The site of implantation in the uterus plays a major role in determining the risk of morbidity and mortality during pregnancy. Xenobiotic influences on the mechanism or site of implantation in primates have not been explored. Evidence from rodent systems suggests that factors altering tubal or uterine motility can alter the spacing of implantation sites as well as endometrial preparation for implantation (*5,25,26*).

## Placentation to Parturition

Early placental development requires an endometrium normally prepared and capable of supporting implantation. Several xenobiotics have been demonstrated to decrease the uterotropic effects of endogenous or exogenous steroids and could alter placentation. These compounds include polycyclic aromatic hydrocarbons and barbiturates as well as certain antibiotics and anticonvulsants (*3,24,27,28*).

Factors altering fetal organogenesis, fetal and placental growth and maturation have been and continue to be characterized by fetal pharmacologists and teratologists and will not be considered here.

## Parturition

Present evidence suggests that fetal hormonal signals control the onset of labor, and drugs that advance and retard the onset of labor have been identified (*29*). After the onset of labor, factors influencing electrical activity of myometrium and electromechanical coupling producing uterine contractions, together with bony structure and degree of extensibility of soft tissues along the birth canal, determine the progression of labor. Although DES has been demonstrated to alter the anatomy of the vagina and cervix, in general the influence of xenobiotics on uterine activity or the birth canal remains uncharacterized.

## Postnatal

Postnatal events including delivery of the placenta, uterine involution, fetal and maternal physiologic and metabolic adaptation and lactation, all focus on resolution of the pregnancy and support and maintenance of the newly born infant. Factors influencing endometrial development, site of implantation and depth of placental penetration into endometrium and myometrium will influence the delivery of the placenta. It is not known if xenobiotics influence primate placentation.

Lactation is discussed by Rogan (*30*); suffice it to say that a variety of xenobiotics can be excreted in breast milk. Additionally, xenobiotics which alter the secretion of breast milk have been identified.

## Reproductive Senescence

Reproductive senescence represents the failure of one or more of the organs, or physiological control mechanisms along the reproductive cycle. Evidence from clinical observations of women as well as studies of experimental animals suggests that declining fertility with age represents combined failure of both the uterus and ovaries. Menopause, a clinical diagnosis, represents a specific subevent in reproductive senescence, the failure of the ovary.

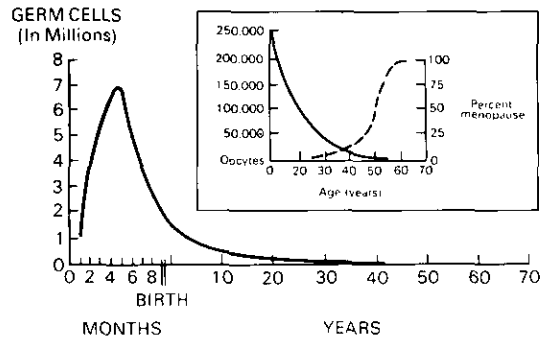


FIGURE 2. Relationship between oocyte number and age in women. The inset indicates the relationship between age, oocyte number and menopause. Reprinted from Mattison and Ross (*24*) with permission.

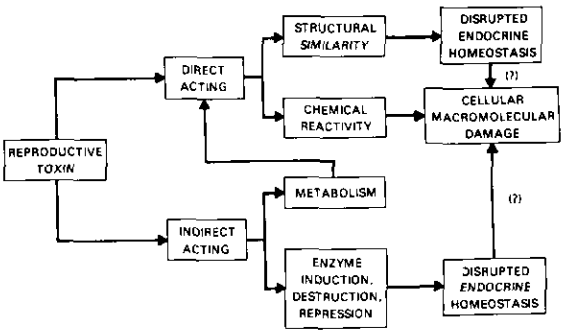


FIGURE 3. Mechanisms of reproductive toxicity. Reprinted from Mattison (*4*) with permission.

Present evidence suggests that the hypothalamus and pituitary remain functional until late in life, long after menopause.

Menopause, a clinical syndrome reflecting diminished ovarian estrogen production, occurs when the ovary is depleted of oocytes (Fig. 2). Factors associated with premature menopause appear to act by destroying oocytes (*3,4,7-9,16,31,32*). Additionally, factors which decrease the formation of oocytes in the fetus are also associated with premature ovarian failure (*15,32*). These data suggest that xenobiotics which block oogenesis or destroy oocytes will produce premature ovarian failure or premature menopause.

## Mechanisms of Reproductive Toxins

In the overview of female reproductive physiology, we identified several reproductive toxins. To understand why these compounds are toxic, we need to define both their site and mechanism of action. One useful scheme for classifying reproductive toxins is illustrated on Figure 3. In this section I will use this scheme to classify the prototype reproductive toxins previously discussed (see Table 2).

### *Diethylstilbestrol*

DES clearly produces some of its effects by virtue of its estrogenic activity (*3,4,33,34*). Additional evidence suggests that the carcinogenic and mutagenic activity of DES may reside in reactive metabolites generated by microsomal monooxygenases or peroxidases (*35-37*).

### Polycyclic Aromatic Hydrocarbons

The polycyclic aromatic hydrocarbons produce their adverse reproductive effects in two ways. Ovarian toxicity and oocyte destruction is produced by reactive electrophilic metabolites generated by metabolism of the parent hydrocarbon (*3,38-40*). Polycyclic hydrocarbons are also inducers of hepatic enzymes including microsomal monooxygenases and transferases involved in steroid metabolism and clearance (*28*).

### Azathioprine and 6-Mercaptopurine

The purine analogs azathioprine and 6-mercaptopurine appear to exert their adverse effects on ovarian development through structural similarity (*41,42*). As the sensitivity of different stages of oogenesis to these purine analogs has not been quantitated, it is not known if the major effect is on primordial germ cells, oogonia or oocytes.

### Galactose

Ovarian failure in women with galactosemia due to deficiencies in uridyltransferase and apparently normal ovarian function in women with galactosemia due to kinase deficiency suggest that toxicity is due to galactose-1-phosphate (*16*). The absence of ovarian toxicity in animals exposed postnatally favors a mechanism involving structural similarity but does not rule out the formation of a reactive metabolite.

### Halogenated Polycyclic Hydrocarbons

This large class of compounds appears to disrupt reproductive function by two mechanisms: induction of hepatic monooxygenases and structural simi-

**Table 2. Mechanisms of action of selected reproductive toxins.**

| | Direct acting | | Indirect acting | |
|---|---|---|---|---|
| Compound | Structural similarity | Chemical reactivity | Metabolism | Enzyme alteration |
| Diethylstilbesterol | * | | * | |
| Polycyclic aromatic hydrocarbons | | * | * | * |
| Purine analogs | | | | |
| Azathioprine 6-mercaptopurine | * | | | |
| Galactose | * | * | * | |
| Halogenated polycyclic hydrocarbons | | | | |
| DDT | * | | | |
| PCB | * | | | * |
| PBB | * | | | * |
| Phytoestrogens | * | | | |
| Barbiturates, anticonvulsants | | | | * |
| Nicotine | * | | | |

larity to estrogens either directly or following metabolism.

## Barbiturates, Anticonvulsants

Certain drugs, notably barbiturates and many anticonvulsants, are good inducers of the hepatic enzymes responsible for steroid clearance. Stimulation of clearance appears to be the major mechanism by which these compounds decrease the effectiveness of oral contraceptives.

## Nicotine

The effect of nicotine on uterine and tubal motility in the primate appears to result from stimulation of nicotinic receptors. This has two effects: release of epinephrine which mimics the effect of nicotine on human utero-tubal motility and stimulation of release of oxytoxin from the posterior pituitary which also alters uterine motility (*5*).

This brief overview of mechanisms indicates that reproductive toxins produce their adverse effects in a wide variety of ways. Complete understanding of both site and mechanism of action of reproductive toxins will be necessary to extrapolate from experimental animal studies to evaluation of human risk. Fortunately a detailed site and mechanism-specific approach will also allow the wide use of a variety of animal model systems tailored directly to the specific physiological and toxicological mechanisms being explored.

# Ovarian Toxicity

In this section we will explore the effects of oocyte destruction on fertility and age of ovarian failure and then consider an experimental animal model system which we have used to investigate mechanisms of oocyte destruction.

In humans and experimental animals ovarian failure occurs when the ovary is depleted of oocytes. The age at which the ovary is depleted of oocytes is controlled by several factors: the number of oocytes formed during oogenesis and the rate of oocyte loss due to ovulation, failure of folliculogenesis, atresia, and toxicity. In general, the number of oocytes lost due to ovulation is small, and in the normal ovary atresia and failure to complete folliculogenesis account for most oocyte loss. However, after treatment with ovarian toxins oocyte loss can be quite extensive.

## Oogenesis and Folliculogenesis

The ovary forms from two separate components: primordial germ cells which originate in an extra embryonic region near the yolk sac and the urogenital ridge (*24*). During organogenesis differential tissue growth brings the primordial germ cells into the hindgut region of the embryo. Primordial germ cells then migrate to the urogenital region from the hindgut. Primordial germ cells proliferate from the time they differentiate until they form oogonia, the stem cells for oocytes.

In the ovary, oogonia proliferate in syncytical groups until folliculogenesis begins. At the onset of folliculogenesis a group of oogonial cells will cease proliferation, the syncytial bridges between oogonial cells are lost and the oogonia enter meiosis. During this period the follicle complex is formed.

The follicle complex is the smallest functional unit of the ovary, consisting of oocyte, granulosa cells, basement membrane and thecal cells. Oocytes require this follicular complex for support. Those oocytes which are not a part of a follicular complex following the completion of folliculogenesis are lost

through extrusion from the surface of the ovary or cell death within the ovary. Xenobiotic or endogenous compounds which block oogenesis or folliculogenesis will decrease the number of oocytes.

## Follicular Growth, Ovulation, Atresia

After sexual maturation, three types of follicular complexes are found in the ovary; resting or primordial follicles, growing follicles and preovulatory follicles (Fig. 4). These three general types of follicle complexes are distinguished on the basis of oocyte size, zona pellucida size, and number of granulosa cells. This classification is important because follicle complexes of different size have different susceptibility to ovarian toxins, and because toxicity to different follicle types may have different effects on fertility and age of ovarian failure.

Follicular growth requires recruitment of follicles from the resting pool into the growing pool. The mechanisms involved in recruitment are poorly understood at the present time. However, the recruitment process may not be completely dependent on gonadotropins. Further growth and ultimate ovulation of the oocyte from the follicle complex requires the appropriate local concentrations of gonadotropins and steroid hormones produced by dynamic interactions between the ovary, hypothalamus and pituitary. Following ovulation, the follicle complex differentiates into the corpus luteum.

Follicle complexes recruited into the growing pool which do not ovulate undergo atresia, the death of the follicle complex. Atresia can occur at any point along the pathway from recruitment to ovulation and occurs because concentrations of gonadotropins and steroid hormones within that follicle complex cannot support its continued proliferation.

## Differential Follicular Toxicity

In experimental animals and humans, follicle complexes have differing sensitivity to ovarian toxins depending on the strains, species, compound and dose schedule used. In rodents single treatment with polycyclic aromatic hydrocarbons, alkylating agents or ionizing radiation destroys predominantly the resting or primordial follicles (*14,31,32,44-46*). In women and nonhuman primates, ionizing radiation and treatment with alkylating agents has its major effect on growing and preovulatory follicle complexes (*7-9,31,32,44*). Mechanisms underlying differential follicle complex sensitivity are not understood.

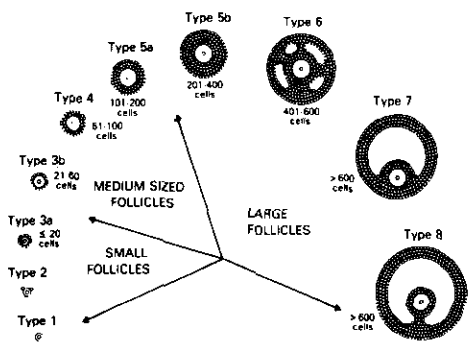


FIGURE 4. Follicle complex classification scheme. Reprinted from Pedersen and Peters (*43*) with permission.

# Oocyte Destruction and Fertility

## Preovulatory Follicle Complex Destruction

In women and nonhuman primates alkylating agents and ionizing radiation destroys growing and preovulatory follicles. As this includes the follicle complex(es) destined to ovulate, this decreases fertility immediately during the period of exposure (Fig. 5). Additionally, destruction of preovulatory follicle complexes destroys the major source of ovarian estrogen synthesis, producing postmenopausal symptoms.

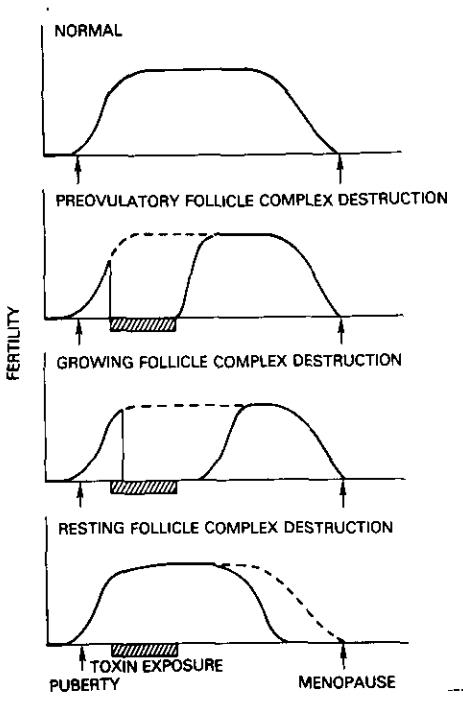


FIGURE 5. Effect of ovarian toxins on fertility. The cross-hatched area represents the period of ovotoxin exposure: (—) the expected fertility; (- -) the normal fertility in absence of toxin exposure.

The dose of cyclophosphamide required to produce permanent amenorrhea varies with age (Table 3). Women treated with substerilizing doses of cyclophosphamide generally resume menses within 18 months following the last treatment course. Resumption of menses is preceded by loss of menopausal symptoms, heralding increasing ovarian estrogen synthesis. The long delay between cessation of treatment and resumption of menses is not completely understood. However, it does not reflect the length of time necessary for follicle complexes to grow to preovulatory size as women with hypogonadotropic hypogonadism can ovulate within 14 days of treatment (*24*).

## Growing Follicle Complex Destruction

At the present time it is not known if there are ovarian toxins which act only on growing follicle complexes. If such compounds did exist, however, their effect on fertility would be somewhat delayed. Existing preovulatory follicles would have to be exhausted before the antifertility effects would be observed (Fig. 5). In primates the length of this delay would be rather short since the follicle destined to ovulate is chosen from the growing pool between 7 and 12 days prior to ovulation (*48*). In practice this suggests that it may not be possible to differentiate between ovarian toxins acting only on growing follicles and those acting only on preovulatory follicles.

## Destruction of Resting Follicle Complexes

Xenobiotics which destroy resting follicle complexes only without altering recruitment, follicular growth or ovulation would have no effect on fertility until all the small follicle complexes were destroyed (Fig. 5). At that point the absence of follicles for recruitment into the growing pool would be followed by depletion of the number of growing follicles and a subsequent failure to form preovulatory follicles. Xenobiotics which destroy only resting follicles would be silent reproductive toxins only identifiable by oocyte quantitation or age of menopause.

**Table 3. Dose of cyclophosphamide required to produce permanent amenorrhea.[a]**

| Age | Total dose, g |
|---|---|
| 20–29 | 20.4 |
| 30–39 | 9.3 |
| 40–49 | 5.2 |

[a]Data from Koyama et al. (*47*).

# Follicle Destruction and Ovarian Failure

Oocyte destruction by xenobiotics or ionizing radiation is associated with premature ovarian failure. As a first approximation it therefore seems reasonable to assume that exposures which decrease the age of menopause, the clinical manifestation of ovarian failure, do so by oocyte destruction.

The age of menopause is directly accessible in either longitudinal, cross section, or case control studies and can rapidly address one facet of reproductive toxicity. One exposure which has been demonstrated to decrease the age of menopause is smoking (Fig. 6). Several well-designed epidemiological studies have demonstrated an earlier age of menopause in smoking women when compared to controls (*5*). The study by Jick et al. shown in Figure 6 demonstrates an inverse dose-response relationship between the number of cigarettes consumed and the age of menopause, suggesting that increasing exposure increases the extent of oocyte destruction (*49*).

The component of cigarette smoke responsible for oocyte destruction in smokers has not been identified. Prime suspects include polycyclic aromatic hydrocarbons and protein pyrolysis products (*5*), compounds demonstrating toxic, mutagenic and carcinogenic properties in several bioassay systems. We have focused on polycyclic aromatic hydrocarbons and have begun to explore the mechanism of oocyte destruction by these compounds.

## Animal Models for Ovarian Failure

Polycyclic aromatic hydrocarbons (PAH) like benzo(a)pyrene (BP) and alkylating agents like

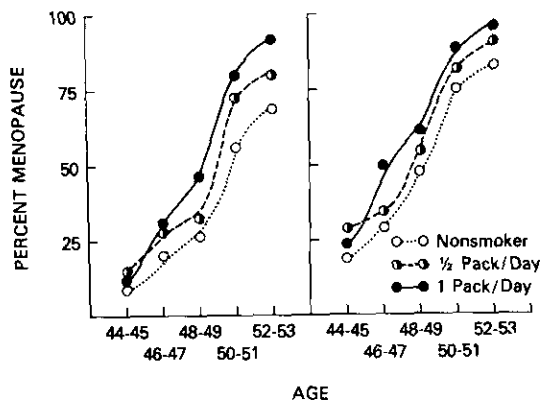


FIGURE 6. Relationship between cigarette consumption and age of menopause: (left) women in the United States; (right) women in seven European countries. Data from Jick et al. (*49*); figure reprinted from Mattison (*3*) with permission.

cyclophosphamide destroy follicle complexes in mouse ovaries. Using this model system we are exploring the age dependence of follicle complex destruction, the role of metabolic activation in follicle complex destruction, the role of detoxification in follicle complex destruction, and strain and species differences in sensitivity to follicle complex destruction.

Using PAH as probe molecules these investigations have demonstrated that follicle complex destruction requires metabolism of the parent compound to a reactive intermediate. Experiments utilizing bilateral and unilateral intraovarian injection have demonstrated that ovarian metabolism is sufficient to produce the reactive ovotoxic matabolites. These data suggest that differences in ovarian metabolic processing of polycyclic hydrocarbons may correlate with age, strain or species differences in follicle toxicity.

Our initial efforts to explore the relationship between follicle toxicity and ovarian PAH metabolism focused on ovarian AHH activity. Differences in inducibility of hepatic aryl hydrocarbon [benzo(a)pyrene] hydroxylase (AHH, EC 1.14.14.1) activity are associated with differences in sensitivity to PAH toxicity, mutagenicity and carcinogenicity in several bioassay systems (*50,51*). Using a murine model system in which inducibility of ovarian AHH activity was inherited as an autosomal dominant trait, we found that resistance to oocyte destruction was inherited as a polygenic trait. These and other data demonstrate that sensitivity to follicle destruction by PAH although dependent on ovarian metabolism is not linked to the *Ah* locus and does not correlate with ovarian AHH activity (*52,53*).

More recent investigations using high pressure liquid chromatographic analysis of benzo(a)pyrene metabolites produced by murine ovarian microsomes suggests that metabolism in the 7,8,9,10-region of BP may be a better measure of the ability of ovarian enzymes to form reactive ovotoxic metabolites (*54,55*). These data correlate well with experiments demonstrating that the 7,8-dihydrodiol-BP is more ovotoxic than BP, suggesting that one follicle toxin generated by ovarian enzymes is the 7,8-dihydrodiol-9,10-oxide-BP, and are also consistent with an extensive literature indicating that an ultimate mutagenic and carcinogenic metabolite of BP is the 7,8-diol-9,10-oxide.

## Conclusions

Xenobiotic compounds can interrupt female fertility at several different sites in the reproductive pathway and by one or more mechanisms. One rapidly ascertainable form of reproductive toxicity is premature or early ovarian failure. Evidence from clinical studies demonstrates that follicle destruction by alkylating agents and other antitumor cytostatics or ionizing radiation produces premature ovarian failure. The demonstration of early cigarette dose-dependent ovarian failure suggests that the age of menopause may be one sensitive indicator of exposure to ovarian toxins. Analysis of the age of menopause in exposed populations in conjunction with experimental animal model studies will help define the utility of this reproductive end point.

Experimental animal model systems will also help define the mechanism of ovarian toxicity. Exploitation of murine models has demonstrated that ovarian metabolism plays a major role in the ovarian toxicity of polycyclic aromatic hydrocarbons. The use of this or other animal model systems may help define the ovarian toxicity of drugs or other xenobiotic compounds. Certainly the ovarian toxicity of complex mixtures of xenobiotic compounds is directly accessible in animal model systems.

Safe, controlled reproduction with the formation of healthy offspring when fertility is desired is the major goal of every couple in the world. Because of this the identification and characterization of xenobiotics which impair reproduction is a crucial issue. Continued and expanded interaction between clinicians, toxicologists, reproductive biologists and epidemiologists is essential to approach this goal of healthy regulated reproduction essential to human kind.

### REFERENCES

1. Symposium on target organ toxicity: gonads, reproductive and genetic toxicity. Environ. Health Perspect. 24: 1-127 (1978).
2. Dixon, R. L. Toxic responses of the reproductive system. In: Toxicology: The Basic Science of Poisons (J. Doull, C. D. Klaassen and M. O. Amdur, Eds.), Macmillan, New York, 1980, pp. 332-365.
3. Mattison, D. R. The effects of biologically foreign compounds on reproduction. In: Drugs During Pregnancy (R. W. Abdul-Karim, Ed.), G. F. Stickley Co., Philadelphia, 1981, pp. 101-125.
4. Mattison, D. R. Drugs, xenobiotics and the adolescent: implications for reproduction. In: Drug Metabolism in the Immature Human (L. F. Soyka and G. P. Redmond, Eds.), Raven Press, New York, 1981, pp. 129-143.
5. Mattison, D. R. The effects of smoking on reproduction from gametogenesis to implantation. Environ. Res. 28: 410-433 (1982).
6. Hunt, V. R. Work and the Health of Women. CRC Press, Boca Raton, 1979, pp. 1-236.
7. Sieber, S. M., and Adamson, R. H. Toxicity of antineoplastic agents in man: chromosomal aberrations, antifertility effects, congenital malformations, and carcinogenic potential. Adv. Cancer Res. 22: 57-155 (1975).
8. Chapman, R. M., Sutcliffe, S. B., and Malpas, J. S. Cytotoxic induced ovarian failure in women with Hodg-

kins disease. J. Am. Med. Assoc. 242: 1877-1884 (1979).
9. Schilsky, R. L., Lewis, B. J., Sherins, R. J., and Young, R. C. Gonadal dysfunction in patients receiving chemotherapy for cancer. Ann. Int. Med. 93: 109-114 (1980).
10. Felton, J. S., Kwan, T. C., Wuebbles, B. J., and Dobson, R. L. Genetic differences in polycyclic aromatic hydrocarbons metabolism and their effects on oocyte killing in developing mice. In: Developmental Toxicology of Energy Related Pollutants (DOE Symposium Series, Vol. 47) (D. D. Mahlum, M. R. Sikov, P. L. Hackett and F. S. Andrew, Eds.), 1978, pp. 1526-1532.
11. Mackenzie, K. M., and Angevine, D. M. Infertility in mice exposed *in utero* to benzo(a)pyrene. Biol. Reprod. 24: 183-191 (1981).
12. Himmelstein-Braw, R., Peters, H., and Faber, M. Influence of irradiation and chemotherapy on the ovaries of children with abdominal tumors. Brit. J. Cancer 36: 269-275 (1977).
13. Himmelstein-Braw, R., Peters, H., and Faber, M. Morphological appearance of the ovarian of leukemic children. Brit. J. Cancer 38: 82-87 (1978).
14. Mattison, D. R., Chang, L., Thorgeirsson, S. S., and Shiromizu, K. The effects of cyclophosphamide, azathioprine and 6-mercaptopurine on oocyte and follicle number in C57BL/6N mice. Res. Commun. Chem. Pathol. Pharmacol. 31: 155-161 (1981).
15. Chen, Y. T., Mattison, D. R., Feigenbaum, L., Fukui, H., and Schulman, J. F. Reduction in oocyte number following prenatal exposure to a high galactose diet. Science 214: 1145-1147 (1981).
16. Kaufman, F. R., Kogut, M. D., Donnell, G. N., Gobelsmann, U., March, C., and Koch, R. Hypergonadotropic hypogonadism in female patients with galactosemia. N. Engl. J. Med. 304: 994-998 (1981).
17. Kupfer, D. Effects of pesticides and related compounds on steroid metabolism and function. Crit. Rev. Toxicol. 4: 83-124 (1975).
18. Kimbrough, R. D. The toxicity of polychlorinated polycyclic compounds and related chemicals. Crit. Rev. Toxicol. 2: 445-498 (1974).
19. Gellert, R. J., Heinrichs, W. L., and Swerdloff, R. S. DDT homologues: estrogen-like effects on the vagins uterus and pituitary of the rat. Endocrinology 91: 1095-1100 (1972).
20. Gellert, R. J., Heinrichs, W. L., and Swerdloff, R. Effects of neonatally administered DDT homologs on reproductive function in male and female rats. Neuroendocrinology 16: 84-94 (1974).
21. Gellert, R. J., and Heinrichs, W. L. Effects of DDT homologs administered to female rats during the perinatal period. Biol. Neonate 26: 283-290 (1975).
22. McLachlan, J. A. (Ed.). Estrogens in the environment. Elsevier North-Holland, New York, 1980, pp. 1-427.
23. Reproductive endocrinology physiology, pathophysiology and clinical management (S. S. C. Yen and R. B. Jaffee, Eds.), W. B. Saunders Co., Philadelphia, 1978, pp. 1-599.
24. Mattison, D. R., and Ross, G. T. Oogenesis and ovulation. In: Laboratory Methods for Evaluating and Predicting Specific Reproductive Dysfunctions. N. Nelson (Ed.), in press.
25. Card, J. P., and Mitchell, J. A. The effects of nicotine administration on deciduoma induction in the rat. Biol. Reprod. 19: 326-331 (1978).
26. Yoshinaga, K., Rice, C., Krenn, J., and Pilot, R. L. Effects of nicotine on early pregnancy in the rat. Biol. Reprod. 20: 294-303 (1979).
27. Welch, R. M., Levin, W., and Conney, A. H. Estrogenic action of DDT and its analogs. Toxicol. Appl. Pharmacol. 14: 358-367 (1969).
28. Welch, R. M., Levin, W., Kuntzman, R., Jacobson, M., and Conney, A. H. Effect of halogenated hydrocarbon insecticides on the metabolism and uterotropic action of estrogens in rats and mice. Toxicol. Appl. Pharmacol. 19: 234-246 (1971).
29. Liggins, G. C. Initiation of parturition. Brit. Med. Bull. 35: 145-150 (1979).
30. Rogan, W. Monitoring breast milk contamination to detect hazards from waste disposal. Environ. Health Perspect. 48: 87-91 (1983).
31. Mandl, A. M. The radiosensitivity of germ cells. Biol. Rev. 3: 288-371 (1964).
32. Ash, P. The influence of radiation on fertility in man. Brit. J. Radiol. 53: 271-278 (1980).
33. Korach, K. S., Metzler, M., and McLachlan, J. A. Diethylstilbesterol metabolites and analogs: new probes for the study of hormone action. J. Biol. Chem. 254: 8963-8968 (1979).
34. Metzler, M. Metabolic activation of carcinogenic diethylstilbesterol in rodents and humans. J. Toxicol. Environ. Health (Suppl.) 1: 21-35 (1976).
35. Metzler, M., and McLachlan, J. A. Peroxidase-mediated oxidation, a possible pathway for metabolic activation of diethylstilbesterol. Biochem. Biophys. Res. Commun. 85: 874-884 (1978).
36. Radiger, H. W., Haenisch, F., Metzler, M., Osch, F., and Glatt, H. R. Metabolites of diethylstilbesterol induce sister chromatical exchanges in cultured human fibroblasts. Nature 281: 392-394 (1979).
37. Barrette, J. C., Wong, A., and McLachlan, J. A. Diethylstilbesterol induces neoplastic transformation without measurable gene mutation at two loci. Science 212: 1402-1404 (1981).
38. Mattison, D. R., and Thorgeirsson, S. S. Gonadal aryl hydrocarbon hydroxylase in rats and mice. Cancer Res. 38: 1368-1373 (1978).
39. Mattison, D. R., and Thorgeirsson, S. S. Ovarian aryl hydrocarbon hydroxylase activity and primordial oocyte toxicity of polycyclic aromatic hydrocarbons in mice. Cancer Res. 39: 3471-3475 (1979).
40. Gelboin, H. V. Benzo(a)pyrene metabolism activation and carcinogenesis: role and regulation of mixed function oxidases and related enzymes. Physiol. Rev. 60: 1107-1166 (1980).
41. Elison, G. S., and Hitchings, G. H. Azathioprine. In: Antineoplastic and Immunosuppressive Agents. II. Handbook of Experimental Pharmacology (A. C. Sartorelli and D. G. Johns, Eds.), New Series Vol. 38/2, Springer Verlag, New York, 1975, pp. 404-425.
42. Paterson, A. R. P., and Tidd, D. M. 6-Thiopurines. In: Antineoplastic and Immunosuppressive Agents. II. Handbook of Experimental Pharmacology (A. C. Sartorelli and D. J. Johns, Eds.), New Series Vol. 38/2, Springer Verlag, New York, 1975, pp. 384-403.
43. Pedersen, T., and Peters, H. Proposal for a classification of oocytes and follicles in the mouse ovary. J. Reprod. Fertil. 17: 555-557 (1968).
44. Baker, T. G. The effects of ionizing radiation on the mammalian ovary with particular reference to oogenesis. In: Handbook of Physiology Endocrinology II. Part I (R. O. Greep and E. B. Astwork, Eds.), American Physiological Society, Washington, D. C., 1976, pp. 349-361.
45. Jull, J. W. Ovarian tumorigenesis methods. Cancer Res. 7: 131-186 (1973).
46. Krarup, T. Oocyte destruction and ovarian tumorigenesis after direct application of a chemical carcinogen (9:10-dimethyl-1:2-benzanthrene) to the mouse ovary. Int. J. Cancer 4: 61-75 (1969).
47. Koyama, H., Wada, J., Nishizawa, Y., Iwanaga, T., Aoki

T., Terasawa, T., Kosaki, G., Yamamoto, T., and Wasa, Cyclophosphamide-induced ovarian failure and its therapeutic significance in patients with breast cancer. Cancer 39: 1403-1409 (1977).
48. diZerega, G. S., and Hodgen, G. D. Folliculogenesis in the primate ovarian cycle. Endocrine. Ref. 2: 27-49 (1981).
49. Jick, H., Porter, J., and Morrison, A. S. Relation between smoking and age of natural menopause. Lancet 1: 1354-1355 (1977).
50. Nebert, D. S., and Jensen, N. M. The *Ah* locus: genetic regulation of the metabolism of carcinogens, drugs and other environmental chemicals by cytochrome P-450 mediated monooxygenases. CRC Crit. Rev. Biochem. 6: 401-443 (1979).
51. Thorgeirsson, S. S., and Nebert, D. W. The *Ah* locus and the metabolism of chemical carcinogens and other foreign compounds. Adv. Cancer Res. 25: 149-193 (1977).
52. Mattison, D. R., and Nightingale, M. S. Oocyte destruction by polycyclic aromatic hydrocarbons is not linked to the inducibility of ovarian aryl hydrocarbon (benzo(a)pyrene) hydroxylase activity in (DBA/2N × C57BL/6N) $F_1$ × DBA/2N backcross mice. Pediat. Pharmacol., in press.
53. Mattison, D. R., and Nightingale, M. S. The biochemical and genetic characteristics of murine ovarian aryl hydrocarbon (benzo(a)pyrene) hydroxylase activity and its relationship to primordial oocyte destruction by polycyclic aromatic hydrocarbons Toxicol. Appl. Pharmacol. 56: 399-408 (1980).
54. Mattison, D. R., West, D. M., and Menard, R. H. Differences in benzo(a)pyrene metabolic profile in rat and mouse ovary. Biochem. Pharmac. 28: 2101-2104 (1979).
55. Mattison, D. R., and Nightingale, M. S. Unpublished data.